

Drosophila neoclavisetae

Picture-wing fly

Species Report

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Drosophila neoclavisetae Species Report, Final Draft

This document was prepared by the staff at the Pacific Islands Fish and Wildlife Office, Honolulu Hawai‘i. We received valuable input and assistance from the following experts: Dr. Karl Magnacca, Oahu Army Natural Resources Program; Dr. Paul Krushelnycky and Dr. Kenneth Kaneshiro, University of Hawai‘i at Mānoa; and Cynthia King, Dr. Will Haines, and Mr. Keahi Bustamente, State of Hawai‘i, Department of Land and Natural Resources, Division of Forestry and Wildlife. We greatly appreciate their guidance and support, which resulted in a more robust report.

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U.S. Fish and Wildlife Service. 2020. Species report for *Drosophila neoclavisetae* (picture-wing fly). Pacific Islands Fish and Wildlife Office, Pacific Islands Interior Region 12, Portland OR. 40 pages.

EXECUTIVE SUMMARY

This Species Report for *Drosophila neoclavisetae* (picture-wing fly) was completed to assess the species' biology, threats, conservation actions, and current status. The U.S. Fish and Wildlife Service (Service) identified the species' ecological requirements for survival and reproduction at the individual, population, and species levels, and identified the factors influencing the species current condition. We used the conservation biology principles of resiliency, redundancy, and representation to assess the overall viability for *D. neoclavisetae*.

Drosophila neoclavisetae is a picture-wing fly in the family Drosophilidae. Historically, the species has been observed two times, in 1969 and 1975, in the wet, montane 'ōhi'a forest of Pu'u Kukui on West Maui between the elevations of 3,400 and 4,500 feet (1,040 and 1,372 meters). The number of individuals remaining is unknown. Adult picture-wing flies are generalist microbivores (microbe eating) and feed upon decomposing plant material. Females oviposit (lay their eggs) in decaying bark and stem tissue of their plant host where the larvae complete development before dropping to the soil to pupate. Pupae develop into adults in approximately one month, and adults sexually mature about one month later. Adults live for 1 to 2 months. Breeding generally occurs year-round, but egg laying and larval development increase following the rainy season as the availability of decaying matter, which picture-wing flies larvae feed on, increases in response to heavy rains. The host of *D. neoclavisetae* is not known, though closely related picture-wing fly species use *Cyanea* spp.

The primary factors that pose serious and ongoing threats to *Drosophila neoclavisetae* and its habitat include limited numbers of individuals and known populations, predation by nonnative wasps and ants, parasitization by nonnative wasps, competition with nonnative invertebrates for host resources, habitat degradation and destruction from nonnative ungulates and nonnative invasive plants, limited availability of host plants, drought and declining humidity, and changes in canopy cover. The extremely limited number of known populations makes the species especially vulnerable to catastrophic events, such as hurricanes or fire.

For the purpose of this Species Report, viability is defined as the ability of *Drosophila neoclavisetae* to persist over time and avoid extirpation. A species is considered viable when there are a sufficient number of self-sustaining populations (resiliency) distributed across the full range of the species (redundancy) and occupying its breadth of habitats to maintain environmental and genetic diversity (representation), so as to allow the species to adapt and persist indefinitely when faced with annual environmental stochasticity and infrequent catastrophic events.

Resiliency of *Drosophila neoclavisetae* is considered extremely low because of low numbers of individuals observed, lack of recently documented populations, predation, parasitization, competition for host plant substrate, and degradation of habitat quality throughout the species' range. The species has extremely low to nonexistent redundancy because the only two populations ever documented were last observed 50 to 55 years ago, despite extensive surveys. The species representation is extremely low because genetic diversity is limited to the species occurrence at Pu'u Kukui in West Maui or perhaps an undocumented, isolated population. Therefore, the current viability of *D. neoclavisetae* is extremely low, if the species is extant.

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SPECIES REPORT OVERVIEW

Introduction

Drosophila neoclavisetae (picture-wing fly), is a member of the family Drosophilidae. The species is endemic to the island of Maui where, historically, it was rare and known only from the Pu‘u Kukui area of West Maui between 3,400 to 4,500 feet (ft) (1,040 to 1,372 meters (m)) in wet forest habitat. The species has only been observed twice: once in 1969 at 4,500 ft (1,372 m) and once in 1975 at 3,400 ft (1,040 m). Despite surveys of approximately 95 percent of its likely range, the species has not been observed since 1975. Picture-wing flies may breed throughout the year, but egg laying generally increases following the rainy season as their larval food supply increases. The specific host of *D. neoclavisetae* is not known, though closely related species such as *Drosophila clavisetae*, use the decaying bark of *Cyanea* spp. as an oviposition and larval host. Female picture-wing flies lay their eggs in decomposing bark, of their host plants, which the larvae use as their feeding substrate. Adult flies are generalist microbivores (microbe eating) and feed upon a variety of decomposing plant material. The picture-wing fly is threatened by low numbers of individuals, predation from non-native invertebrates such as yellow-jacket wasps and ants, parasitization by nonnative wasps, and competition for resources from crane flies. The species is also threatened by factors that harm host plant populations or alter the decay cycle of its host plants and degrade its habitat. These threats include browsing or trampling of host plants by pigs; damage or consumption of host plant seeds by rats; invasive nonnative plants; drought; changes in overstory canopy and the microclimate of the understory; fire, and hurricanes.

This Species Report, prepared by the Pacific Islands Fish and Wildlife Office, summarizes the biology and current status of *Drosophila neoclavisetae*. The Species Report provides an in-depth review of the species’ biology, threats, and conservation actions that influence viability, followed by an evaluation of current species status and viability.

The intention of the Species Report is for it to be easily updatable and to support the functions of the Service’s Endangered Species Program. The Species Report is a living document upon which other documents, such as recovery plans and 5-year reviews, will be based.

Regulatory History

Drosophila neoclavisetae was listed as endangered on May 9, 2006 (USFWS 2006a) and a recovery outline including the species was published in August 2006 (USFWS 2006b). Ecosystem-based critical habitat totaling 584 acres (237 hectares (ha)) was designated December 8, 2008 for *D. neoclavisetae* on the island of Maui (USFWS 2008, entire). On April 8, 2010, the Service published a notice of the initiation of the 5-year status review (USFWS 2010) and a 5-year review was published on August 28, 2012 (USFWS 2012). On February 12, 2016, the Service published a notice of the initiation of the 5-year status review (USFWS 2016a) and a 5-year review was published on June 24, 2018 (USFWS 2018b). The Recovery Outline for Hawaiian Multi-Island Species includes *D. neoclavisetae* and was published June 30, 2020 (USFWS 2020).

Methodology

We used the best scientific and commercial data available to us, including peer-reviewed literature, grey literature (government, academic, business, and industry reports), and expert elicitation.

This Species Report assesses the ability of *Drosophila neoclavisetae* to maintain viability over time. Viability is the ability or likelihood of the species to maintain populations over time, i.e., likelihood of avoiding extinction. To assess the viability of *D. neoclavisetae*, we used the three conservation biology principles of resiliency, redundancy, and representation, or the “3Rs” (Figure 1; USFWS 2016b, entire). We will evaluate the viability of *D. neoclavisetae* by describing what the species’ requires to be resilient, redundant, and represented, and comparing that to the status of the species in its current condition based on the most recent information available.

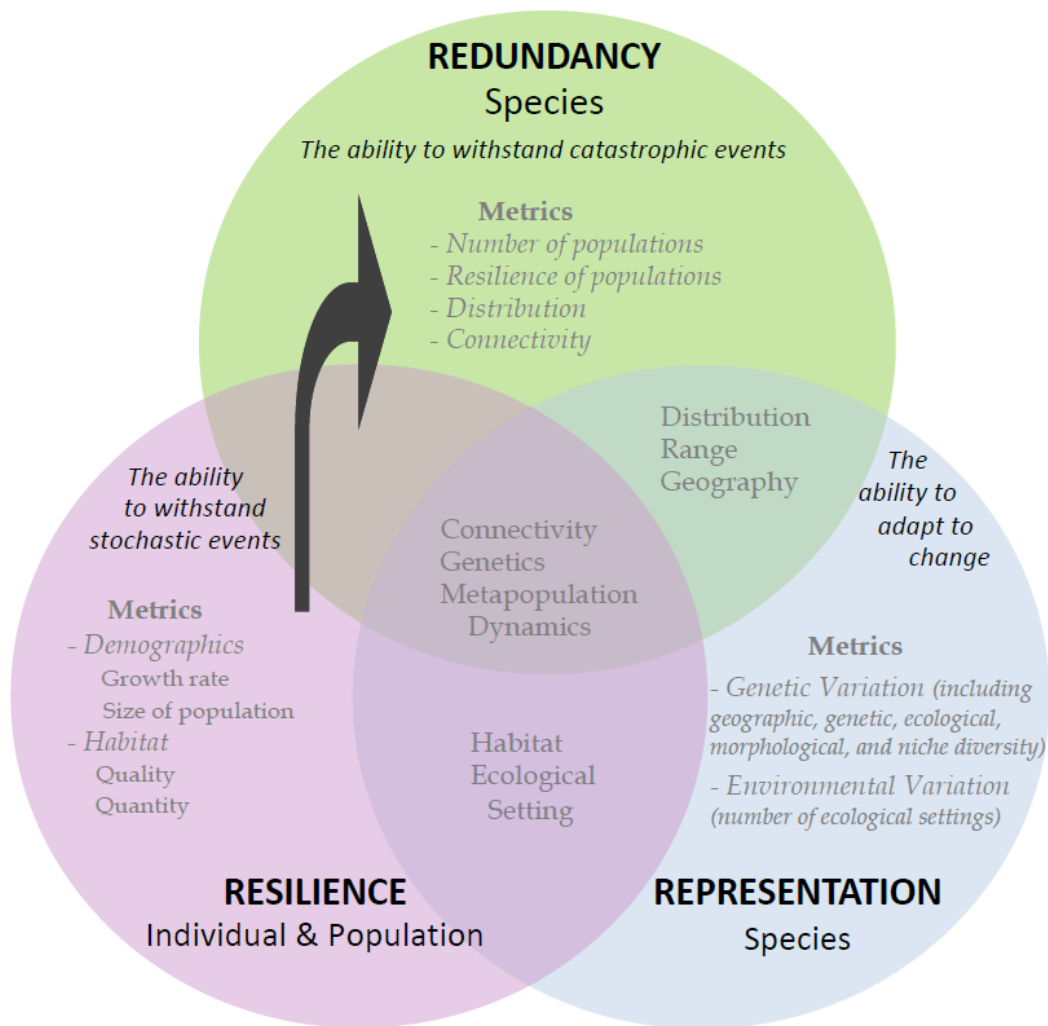


Figure 1. The three conservation biology principles of resiliency, redundancy, and representation (3Rs) used to assess species viability.

Definitions

Resiliency is the capacity of a population or a species to withstand the extreme limits of normal year-to-year variation in environmental conditions such as temperature and rainfall extremes, and unpredictable but seasonally frequent perturbations such as fire, flooding, and storms (i.e., environmental stochasticity). Quantitative information on the resiliency of a population or species is often unavailable. However, a population or species found within a known area over an extended period (e.g., seasons or years) is likely to be resilient to current environmental stochasticity. If quantitative information is available, a resilient population or species will show enough reproduction and recruitment to maintain or increase the numbers of individuals in the population or species, and possibly expand the range of occupancy. Thus, resiliency is positively related to population size and growth rate and may be influenced by the connectivity among populations.

Redundancy is having more than one resilient population distributed across the landscape, thereby minimizing the risk of extinction of the species. To be effective at achieving redundancy, the distribution of redundant populations across the geographic range should exceed the area of impact of a catastrophic event that would otherwise overwhelm the resilient capacity of the populations of a species. In the report, catastrophic events are distinguished from environmental stochasticity in that they are relatively unpredictable and infrequent events that exceed the more extreme limits of normal year-to-year variation in environmental conditions (i.e., environmental stochasticity), and thus expose populations or species to an elevated extinction risk within the area of impact of the catastrophic event. Species redundancy is conferred when the geographic range of the species exceeds the impact area of a catastrophic event. In general, a wider range of habitat types, a greater geographic distribution, and connectivity across the geographic range will increase the redundancy of a species and its ability to survive a catastrophic event.

Representation is having more than one population of a species occupying the full range of habitat types used by the species. Alternatively, representation can be maintaining the breadth of genetic diversity within and among populations, in order to allow the species to adapt to changing environmental conditions over time. The diversity of habitat types, or the breadth of the genetic diversity of a species, is strongly influenced by the current and historic biogeographical range of the species. Conserving this range should consider historic latitudinal and longitudinal ranges, elevation gradients, climatic gradients, soil types, habitat types, seasonal condition, etc. Connectivity among populations and habitats is also an important consideration in evaluating representation.

Species viability is derived from the combined effects of the 3Rs. A species is considered viable when there are a sufficient number of self-sustaining populations (resiliency) distributed over a large enough area across the range of the species (redundancy) and occupying a range of habitats to maintain environmental and genetic diversity (representation) to allow the species to persist indefinitely when faced with annual environmental stochasticity and infrequent catastrophic events. The 3Rs share ecological factors such as connectivity among habitats across the range of the species. Connectivity sustains dispersal of individuals, which in turn greatly affects genetic diversity within and among populations. Connectivity also provides access to the full range of habitats normally used by the species and is essential for re-establishing occupancy of habitats following severe environmental stochasticity or catastrophic events (see Figure 1 for more

examples of overlap among the 3Rs). Another way the three principles are inter-related is through the foundation of population resiliency. Resiliency is assessed at the individual and population level; redundancy and representation are assessed at the species level. Resilient populations are the necessary foundation needed to attain sustained or increasing Representation and redundancy within the species. For example, a species cannot have high redundancy if the populations have low resiliency. The assessment of viability is not binary, in which a species is either viable or not, but rather on a continual scale of degrees of viability, from low to high. The health, number, and distribution of populations were analyzed to determine the 3Rs and viability. In broad terms, the more resilient, represented, and redundant a species is, the more viable the species is. The current understanding of factors, including threats and conservation actions, will influence how the 3Rs and viability are interpreted for *Drosophila neoclavisetae*.

SPECIES ECOLOGY

Species Description

The genus *Drosophila*, in the order Diptera (referring to having only two wings), belongs to the family Drosophilidae, from the Greek *drosos* (dew, plus *philios*, loving) (Hardy 1965, p. 13). Of the 1,665 known *Drosophila* species, nearly 1,000 are endemic to Hawai‘i (Kaneshiro 1997, p. 452; Kang et al., 2016, entire; O’Grady and DeSalle 2018, p. 14). The species of Drosophilidae endemic to the Hawaiian archipelago were previously described in five genera: *Antopocerus*, *Ateledrosophila*, *Drosophila*, *Idiomya*, and *Nudidrosophila* (Kaneshiro 1976, pp. 255-257). However, several lines of evidence indicate all species are part of only two lineages: *Drosophila* and *Scaptomyza* (Hardy 1965, entire; Carson and Kaneshiro 1976, entire; Kaneshiro et al., 1995, pp. 59-60). The genus *Drosophila* has been further separated into groups of species (Kaneshiro 1988, entire; Hardy and Kaneshiro, 1975, entire; Hardy and Kaneshiro, 1979, entire; O’Grady et al. 2010, entire). Currently, eight species groups are recognized in the Hawaiian *Drosophila*: picture wing, modified mouthparts, modified tarsus, antopocerus, ateledrosophila, nudidrosophila, haleakalae, and rustica (O’Grady and DeSalle (2018, p. 14). *Drosophila neoclavisetae* is in the picture-wing species group.

While there is uncertainty as to whether *Drosophila* species in Hawai‘i arose from a single or multiple introductions to the Hawaiian Island chain over 25 million years ago, DNA analyses, to date, agree that the Hawaiian picture wing-fly group within the *Drosophila* genus, arose from a single colonization event (Carson 1997, pp. 343-345; Carson and Kaneshiro 1976, pp. 316; Davis 2000, entire; Kaneshiro and Kaneshiro 1995, p. 1; Magnacca et al. 2008, p.1). Island formation and subsequent colonization by the Hawaiian *Drosophila* species has progressed from north to south as islands formed, emerge above sea level, and then subsided. A more detailed description of the geologic history of the Hawaiian Islands can be found in Harrington et al. (2020, entire).

The picture-wing group represents biological diversity within a single large, closely related group of species and is a prime example of adaptive radiation (Foote and Carson 1995, pp 368-369; Kaneshiro 1976, p. 276; Magnacca and Price 2015, entire). Adaptive radiation refers to the evolution of an ancestral species, which was adapted to a particular way of life, into many diverse species, each adapted to a different habitat (Craddock and Kabysellis 1997, p. 488). Perkins (1913, p. xl) observed "the tendency of island creatures to limit their range and to specialize their habits" foreshadowing the concept of adaptive radiation and the intimate

association between each organism and its microenvironment (Craddock and Kambysellis 1997, p 476; Kaneshiro 2006a, entire).

Within the *Drosophila* genus, the picture-wing fly group likely branched off from the *Scaptomyza* about 15 to 23 million years ago (Magnacca et al. 2008, p. 2; Magnacca and Price 2015, p. 233). The picture-wing fly group consists of 112 known species, most of which are quite distinct from each other in morphology, pigmentation, and behavior (Carson 1997, pp. 344-345; Carson and Kaneshiro 1976, pp. 328, 338; Edwards et al. 2007, entire).

Historically, inversion patterns of the giant polytene chromosomes of 106 picture-winged species and morphological comparisons have been used to develop a pattern of relationships within the picture-wing fly group (Carson, 1992, entire; Kaneshiro et al. 1995, entire). As a result, the picture-wing flies are further subdivided into three species groups or clades: *adiastola*, *planitibia*, and *grimshawi*. *Drosophila neoclavisetae* is in the *adiastola* group (Carson and Kaneshiro 1976, pp. 322–327; Edwards et al. 2007, entire; Kaneshiro et al 1995, p. 64). The banding pattern of the giant polytene chromosomes of *D. neoclavisetae* and *D. clavisetae* are identical (Carson 1990, entire). As genetics advanced, the species groups, including *adiastola*, were further divided into subgroups based on the likelihood that the species share a common ancestor, and thus are considered closely related. Presently, *D. neoclavisetae* shares the subgroup *clavisetae* with closely related *D. clavisetae* (East Maui) and two other picture wing fly species from other islands: *D. neogrimshawi* (O‘ahu), and *D. spectabilis* (Maui, Moloka‘i, and Lāna‘i) (Figure 2; Magnacca and Price 2015 p. 228). *D. neoclavisetae*, from West Maui, and *D. clavisetae*, from East Maui, are almost identical in morphology and are likely sister species. Closely related species likely arose through speciation of a founder from an older Hawaiian island (Kaneshiro et al. 1995, pp. 64-68; Lim and Marshall 2017, entire).

Drosophila neoclavisetae was originally described by Perreira and Kaneshiro (1990, pp. 81–83) from specimens collected at Pu‘u Kukui, West Maui, in 1969. It was named for its similar characteristics with *D. clavisetae* from East Maui. These two species are almost identical in morphology. Both species are similar in wing and thorax markings. Based on Perreira and Kaneshiro (1990, pp. 81–83), *D. neoclavisetae* is between 0.24 and 0.25 inches (in) (6.0 and 6.4 millimeters (mm)) in length, with wings 0.26 to 0.28 in (6.5 to 7.0 mm) long. It is distinguished by its amber brown head and yellow face, with the middle portion raised to form a prominent ridge. The thorax is predominantly reddish brown with a distinct brown median stripe, bordered on each side by two brown stripes. The legs are yellow, with brown on the femora and a distinct brown band on the tips of the tibiae. The wings are broad and rounded, more than twice as long as wide, and with the front portion covered with brown markings and large clear spots tinged light yellow-brown. It shares with *D. clavisetae* an extra (third) cross-vein in the wing, which distinguishes both *D. neoclavisetae* and *D. clavisetae* from the other species of the *adiastola* group. The third crossvein is likely an adaption that provides mechanical support for larger wings (Edwards et al. 2007, p. 4). The abdomen is dark brown and black with numerous long hairs on the hind segments of the male. These hairs are rounded and not flattened like those seen on *D. clavisetae*. For a more detailed description of the species, see Perreira and Kaneshiro (1990, pp. 81–83).

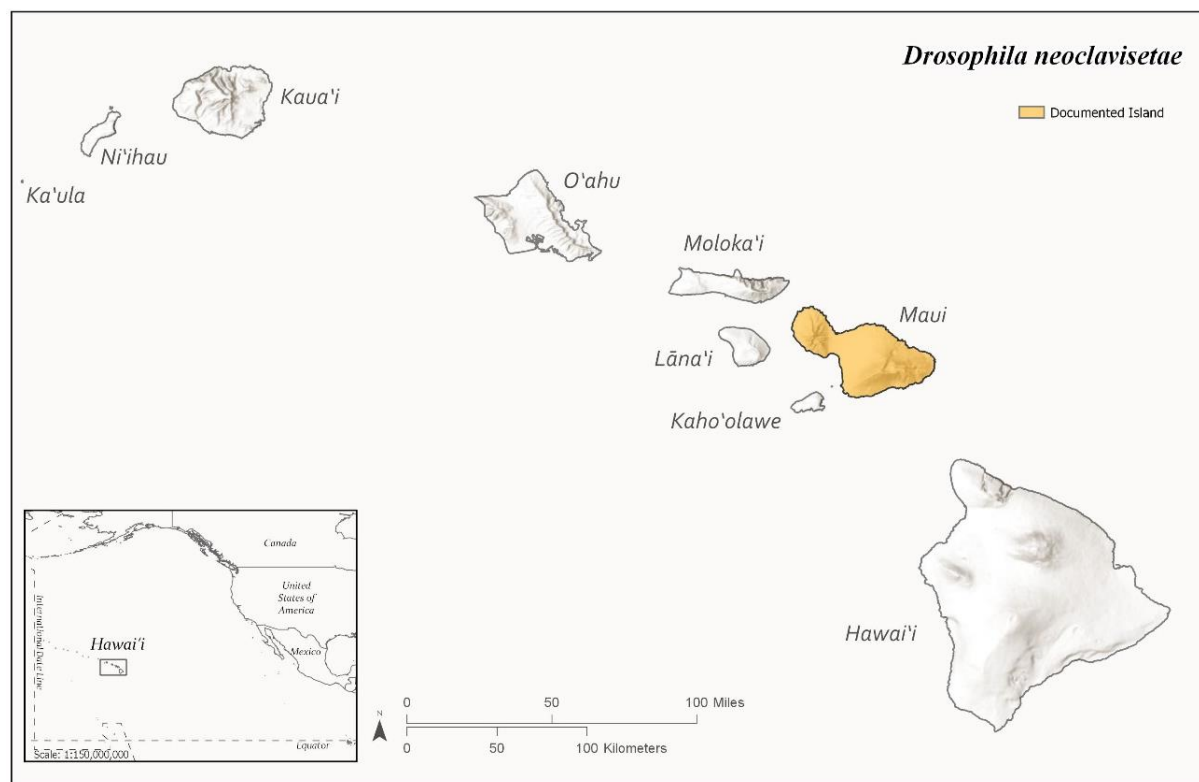


Figure 2. Range of *Drosophila neoclavisetae* within the Hawaiian Island archipelago. Map compiled from Esri (2020) and Hawai'i Statewide GIS Program (2020) data sets.

Habitat and Diet

Drosophila neoclavisetae is an endemic known only from the wet montane *Metrosideros polymorpha* (‘ōhi‘a) forests, between the elevations of 3,400 to 4,600 ft (1,040 to 1,400 m) on West Maui (Figure 2 and Figure 3) (Perreira and Kaneshiro 1990, p. 83; Kaneshiro 2005 in litt., entire). In general, the wet montane ecosystem has an annual precipitation of greater than 75 in (191 cm). This rare picture-wing fly species has only been documented two times, the last time occurring in 1975 (Figure 3) (Perreira and Kaneshiro 1990, p. 83; Kaneshiro and Kaneshiro 1995, p. 26; Kaneshiro 2005 in litt., entire).

Many endemic Hawaiian *Drosophila* species are highly host plant specific (Magnacca et al. 2008, entire; Montgomery 1975, entire). Adult *Drosophila neoclavisetae* are opportunistic microbivores and feed upon a decomposing plant material; however, oviposition (egg laying) and subsequent larval development are limited to specific plant hosts (Montgomery 1975, entire; Kaneshiro and Kaneshiro 1995, p. 26; Science Panel 2005, p. 26; Magancca 2008, entire). The *adiastola* group almost exclusively utilizes lobeliads as hosts (Magnacca et al. 2008, entire; Magnacca and Price 2015, p. 236). The host plant of *D. neoclavisetae* has not yet been confirmed, although it is almost certainly associated with *Cyanea* sp. (family Campanulaceae) (Kaneshiro and Kaneshiro 1995, p. 20; Science Panel 2005, p. 26). Both collections of *D. neoclavisetae* occurred within a small patch of *Cyanea* spp. and all of the picture-wing fly species in the *adiastola* species subgroup use *Cyanea* spp. or other plants in the family Campanulaceae as oviposition (laying of eggs) and larval substrate (Montgomery 1975, p. 83; Magnacca et al. 2008, p. 11). Two *Cyanea* species are found within the known range of *D.*

neoclavisetae: *Cyanea kunthiana* (hāhā) and *Cyanea macrostegia* (hāhā) (USFWS 2008 p. 73801; USFWS 2012 p. 6). The endangered *C. kunthiana* is known from East and West Maui Mountains. ON West Maui, *C. kunthiana* occurs at the junction of Honokōwai, Hāhākea, and Honokōhau valleys, an area from which *D. neoclavisetae* is known (USFWS 2016a, p. 17800). *C. macrostegia* is abundant and is considered stable in population number (Science Panel 2005, p. 20). *C. macrostegia* is unevenly distributed across the wet montane habitat of *D. neoclavisetae* (Bartlett 2006, entire; Hanson 2006, entire).

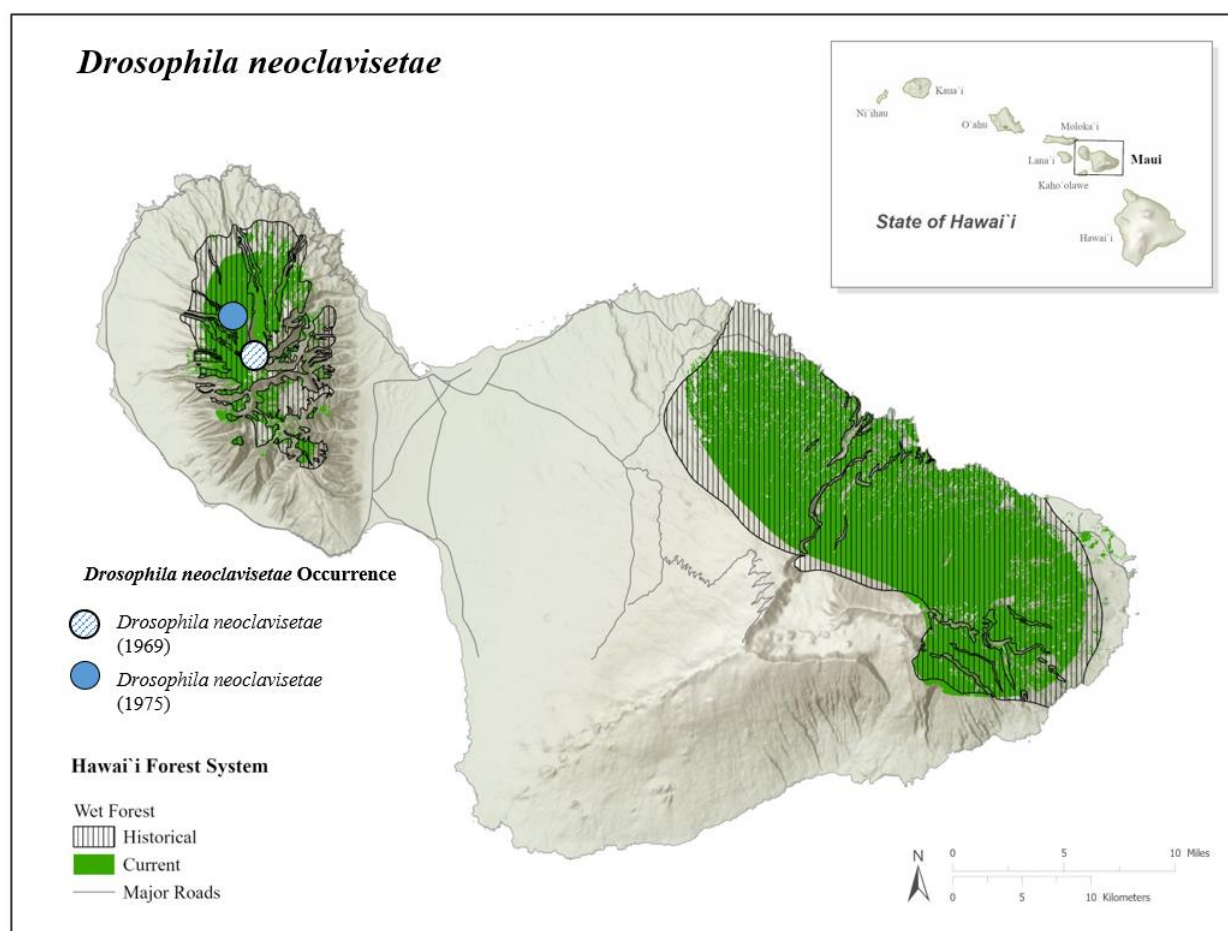


Figure 3. Known occurrence of *Drosophila neoclavisetae* in 1969 and 1975. Map adapted from Clark et al. 2020, entire, and Reeves and Amidon 2018, entire.

The picture-wing fly species in the *adiastola* group use decaying bark and stems of their plant hosts for oviposition and larval development (Montgomery 1975, entire; Magnacca et al. 2008, entire). Availability of a suitable stage of substrate is necessary for egg deposition to occur. If the female does not find a suitable host and substrate condition, she will not typically lay eggs. At least one of the likely host species, *Cyanea kunthiana*, is endangered (USFWS 2013b, entire). Populations of these plant hosts are vulnerable to threats such as ungulates and rats that can leave them isolated or sparsely distributed without active ungulate control (Science Panel 2005, pp. 19–20; USFWS 2013a, entire; USFWS 2013b, entire; USFWS 2020, entire). Such isolation of host plants can affect breeding success of the picture-wing flies.

Life Cycle and Demographics

The general life cycle of Hawaiian Drosophilidae is typical of most flies: after mating, females lay 50 and 200 eggs in a single clutch from which larvae (immature stage) hatch after 5 to 7 days (Science Panel 2005, p. 4). The larvae complete development in decaying tissue of a host plant in two to four weeks (Science Panel 2005, p. 4, 26). As the larvae grow, they molt (shed their skin) through three successive stages (instars). When fully grown the larvae drop to the ground to pupate (Montgomery 1975, pp. 65–103; Spieth 1986, p. 105). Pupae develop into adults in approximately 1 month, and adults sexually mature 1 month later. Adults live for 1 to 2 months (Borror et al. 1989, p. 738). Breeding generally occurs year-round, but egg laying and larval development increase following the rainy season as the availability of decaying matter, on which picture-wing flies feed, increases in response to heavy rains.

The picture-wing flies have been referred to as the “birds of paradise” of the insect world because of their relatively large size, colorful wing patterns, and the males' territorial defense behaviors and elaborate courtship displays (Kaneshiro and Kaneshiro 1988, p. 258). In general, breeding for picture-wing flies involves two stages, lekking (i.e. establishing a mating territory) and mating. It has been suggested that the lengthy and conspicuous lekking and courtship behaviors of the picture-wing flies make them more vulnerable to predation (Kaneshiro 2006b in litt., entire).

Individual picture-wing flies show a level of aggressiveness towards one another except when they are on the feeding and ovipositional sites. At the feeding sites, there is a complete absence of antagonistic behavior (Spieth 1986, pp. 105-106). Except during lekking, courtship and mating, adults are generally secretive and solitary during the daylight hours (Spieth 1986, p. 103). The flies ignore light to moderate precipitation, but disappear during intense rainfall, apparently seeking refuge (Spieth 1986, p. 103). In their movement through the forests, if two individuals come into close proximity with one another, antagonistic behavior occurs and one or both individuals flee by flying laterally or diving downward (Spieth 1986, p. 103).

During lekking, males fight among themselves for the best mating territory, or lek, and establish a dominance hierarchy (Kaneshiro and Kaneshiro 1995, p. 4). The lek is never on the egg deposition site (Heed 1971). Rather, males of the *adiastola* group select smooth, dark colored areas on tree trunks, the undersides of branches, occasionally stems of tree fern fronds or shrubs as lek sites. Areas are typically about 2 to 3 in (5 to 7.6 cm) or less in diameter, usually situated 1 to 4 ft (0.3 to 1.2 m) above ground, invariably in dense vegetation where the light intensity is low and surrounded by foliage (Spieth 1978, p. 439). The lek is near a food and oviposition site (Spieth 1986, p. 103, 105-106). Male picture-wing flies use different techniques to ward off other males competing for females. For example, some butt heads, similar to bighorn sheep, to win the territory; some species joust; others grasp one another with legs and wings and wrestle; and still others create an intimidating, buzzing roar with muscles from its abdomen (Spieth 1974, entire; Spieth 1981, entire; Spieth 1986, entire). The loser flees (Spieth 1981, p. 925).

Once the male establishes his lek, the male advertises his sexual readiness. When a male has attracted the female, he performs a detailed choreography of courtship behaviors for females visiting the lek. The male *Drosophila neoclavisetae*, like the closely related *D. clavisetae*, have what has been described as one of the most bizarre courtship dances in the Drosophilidae

(Kaneshiro and Kaneshiro 1975 in litt., p. 24). According to Spieth 1978 (pp. 441–442), the male extends and thrusts his face towards the female, spreads his wings 45 degrees laterally and subsequently extends them from 100 to 110 degrees and rotates them to vertical. He then curls his abdomen 180 degrees upward and forward until the tip is above his head or thorax pointing forward. He then vibrates the tip of the abdomen rapidly up and down secreting a drop of fluid from his abdomen in front of the female. The female then touches this droplet, obtaining chemosensory information (Spieth 1978 pp. 441–442; Anholt et al., 2020, p. 54). The wing waving and vibration can go on for many minutes.

If he does not convey the right moves and messages to her, she leaves without mating, despite his lengthy courtship dance (Spieth 1978, p. 437). Each species also has its own mating ritual; some include dancing around the female, wing displays similar to a peacock, vibrating his wings at a specific pitch, licking the female, or dousing the female with pheromone, or, as in the case of the *adiastola* group, placing the male's head under the female's wing (Spieth 1974, entire; Spieth 1978, pp 439–442; Spieth 1981, pp. 815-816; Spieth 1986, pp. 103-105; Anholt et al. 2020, entire).

In a newly established population, there may be strong selection for less discriminating females (Kaneshiro 1988, entire). In a population with limited individuals, highly discriminating females may never encounter males that are able to satisfy their courtship requirements, whereas females that are less discriminating will be most likely to mate and leave progeny (Kaneshiro 1980, pp. 438-440; Kaneshiro 1988, pp. 261-262; Kaneshiro 2004, entire). Such new founding events may correlate with plant host availability cycles and create a shift in the distribution of female mating types towards those that are less discriminating (Kaneshiro 1988, entire). Such a shift in the mating system provides a means for genetic recombination (Kaneshiro 2004, entire).

Drosophila spp. use chemosensation (i.e., chemical detection using various sensory organs) for detecting food, avoiding toxicants, as well as for courtship and mating. Strong behavioral differences related to courtship and reproduction may be driving genome divergence of closely related species (Kang et al. 2016, p. 1487). The genomic differences in genes related to smell and taste reception seem to play a major role in adaptive radiation in the picture-wing flies (Kang et al 2016, entire).

Individual Needs

The primary factors that pose serious and ongoing threats to *Drosophila neoclavisetae* individuals include: low numbers; predation by nonnative wasps and ants; parasitization; competition with nonnative invertebrates for host resources; habitat degradation and destruction from nonnative ungulates and nonnative invasive plants; rat predation on host plants, seeds, and picture-wing fly larvae; drought and declining humidity, and increased risk of fire.

An individual adult *Drosophila neoclavisetae* survives about 1 to 2 months. The adult is an opportunistic generalist, feeding on microbes in decaying plant material for survival. For adult survival, host plant species is not likely a limiting factor. Rather, the decay status of the plant material and the presence of suitable microbes on the decaying material are primary factors in adult survival, in the absence of anthropogenic-associated threats. Humidity and other factors influence the availability and suitability of this resource.

Egg laying and larval development generally increase following the rainy season as the availability of decaying matter increases in response to heavy rains. For oviposition to occur, an adult female that has successfully found a mate and bred must find a suitable host substrate. This need may restrict a female to particular areas. It is expected that many of the picture-wing fly species probably travel some distance to reach host plants (around 100 meters has been estimated), especially within contiguous forest (Science Panel 2005, p. 5). However, species feeding upon highly isolated host plants probably do not move much between plant populations (Science Panel 2005, p. 5). *Drosophila neoclavisetae* egg and larvae development both require decaying bark or stems of its host plant, believed to be *Cyanea* spp. (Montgomery 1975, p. 83; Kaneshiro and Kaneshiro 1995, p. 20; Science Panel 2005, p. 26; Magnacca et al. 2008, entire). The substrate, selected by the female, must first provide suitable environment for eggs laid to hatch. Second, the substrate needs to supply adequate nourishment for the larvae to develop to pupation. Provided suitable environmental conditions (high humidity and presumably cool temperature) exist and threats were absent, the adult individual will emerge about 1 month after pupation.

Population Needs

Hawaiian picture-wing flies, including *Drosophila neoclavisetae*, evolved in isolated habitats, resulting in tremendous speciation; as a result, small population size may be less of a threat than small habitat size, decreasing availability of suitable oviposition and larval host substrates, and predation (Science Panel 2005, entire; Kaneshiro 2004, entire). Resiliency is the capacity of an individual or population to withstand stochastic disturbance events. The survival rate of *Drosophila neoclavisetae*, population demographics, and growth rate needed to sustain a population in the presence of threats are unknown. For *D. neoclavisetae* to be abundant, individuals must reproduce. This requires mating and having a stable to positive population growth rate. Thus, we base resiliency of *D. neoclavisetae* on population size (abundance), host availability, and habitat quality.

For picture-wing flies, population growth requires a series of actions. After the adult males emerge from their pupal stage, it takes them approximately one month before they are sexually mature. Male flies must practice their courtship displays in the presence of other males before they can be successful at courting a female fly. This behavior along with defending their lek territory and the actual courtship dance exposes them to predation from nonnative wasps (Kaneshiro and Kaneshiro 1995).

Sexual selection is very important to these species (Carson 2002, entire; Kaneshiro 1980, entire; Kaneshiro 2004, entire; Spieth 1986, p. 103-105). Each species has its own elaborate courtship actions and songs (Science Panel 2005, p. 4-5; Hoy et al., 1988). Because sexual selection is so important, the establishment of a territory by a male is not a guarantee of breeding (Science Panel 2005, p. 5). If the male does not convey the right moves and messages during courtship, the female leaves without mating (Kaneshiro 1980, pp. 438-440; Kaneshiro 1988, pp. 261-262; Kaneshiro 2004, entire). The absence of a direct correlation of male's plant host selection for its lek relegates host selection mainly to the female after she is fertilized (Heed 1971).

In order to oviposit, the *Drosophila neoclavisetae* female needs a stable and constant supply of suitable host plant substrate for oviposition, egg hatching and larval development (Magnacca et al. 2008, p. 14; Magnacca 2012a in litt., entire; Montgomery 1975, pp. 83, 96–97). If she fails to find the right substrate, she will not lay eggs. The egg laying behavior of the female picture-wing fly determines the larval substrate specificity. Additionally, the correct life stage of the host plant is critical in influencing egg laying behavior (Science Panel 2005, p. 4). Although breeding generally occurs year-round, egg laying and larval development increase following the rainy season as the availability of decaying matter typically increases. Species reliant on specific host plants are vulnerable to extinction if the plant host becomes too rare or scattered, even if the plant does not itself become extinct (Magnacca et al. 2008, p. 32).

The species is known only from the Pu‘u Kukui area in the wet montane forests at the elevations of 3,400 and 4,600 ft (1,040 and 1400 m) (Perreira and Kaneshiro 1990, p. 83; Kaneshiro 2005 in litt., entire; Magnacca 2012b in litt., entire; Magnacca 2019 in litt., entire). Host plants of *Drosophila neoclavisetae* are susceptible throughout their range to damage from ungulates, mainly pigs, in unfenced areas and to habitat degradation caused by invasive plants. The picture wing fly population is also sensitive to changes in overstory, understory, and drought, which results in reduced humidity in the flies’ microclimate (Science Panel, 2005 in litt., p. 26; Magnacca 2008, entire). Species like *D. neoclavisetae* that rely on decaying substrates, are particularly sensitive to bottlenecks in host populations due to their dependence on older, senescent or dying plants (Magnacca et al. 2008, p. 32). Microclimate alteration, such as opening of the canopy or understory, adversely affects the flies, either directly through increased desiccation or by causing host plants to dry more rapidly and rot differently (Magnacca 2012a in litt., entire). Reduced humidity changes the decay process of the host plants and alters substrate availability for the picture-wing fly.

Even when conservation measures are implemented (e.g., fencing out feral ungulates from sections of native forest or controlling alien weeds), there may be a gap between the death of older hosts and the maturation of new seedlings to the point where they are able to provide sufficient resources for *Drosophila neoclavesetae* populations to survive (Magnacca et al. 2008, p. 32). Other picture-wing fly species that are dependent on more readily renewable substrates, such as those breeding in leaves, have been shown to persist for years at a single tree (Heed, 1968 as cited in Magnacca et al. 2008, p. 32) and may not be subject to the same bottleneck pressures that impact decaying bark-specific taxa.

Resiliency depends on absence of threats. The greatest known threats to picture-wing fly populations are predation by wasps and ants, parasitization by nonnative wasps, and competition for resources. The lengthy and conspicuous lekking and courtship behaviors of *Drosophila neoclavisetae* adults and their relatively large size, leave the species vulnerable to predation by yellow-jacket wasps (Kaneshiro 2006b in litt., p. 2). Other nonnative wasps may indiscriminately sting the larvae of *D. neoclavisetae* resulting in its mortality. Eggs, larvae, and pupae are vulnerable to predation by ants. Competition for limited larval substrate can exhaust larval food resources. Tipulid flies can exhaust decaying bark food resources, which affects both the probability of *D. neoclavisetae* larval survival and the body size of adults. This can result in reduced adult fitness, fecundity, and lifespan.

The limits on plant host populations are, most likely, less significant than the impacts of low population numbers of this picture-wing fly. This historically rare picture-wing fly is only known from locations near Pu‘u Kukui in West Maui where it was documented in 1969 and again in 1975 (Magnacca 2012b in litt., entire; Magnacca 2012c in litt., entire; Magnacca 2019 in litt., entire). The species has not been observed since, despite surveys. This infers a lack of positive population growth. Accordingly, *D. neoclavisetae* resiliency requires quality habitat without threats, in addition to host availability, abundant individuals in each population, and an increasing number of populations in the wild.

Species Needs

For a species to thrive, it needs to have multiple stable to growing populations occurring throughout its range representing all known ecological and species-specific genetic diversity. Redundancy is the ability of *Drosophila neoclavisetae* to withstand catastrophic events and is measured by the number of populations (redundancy/duplication), distribution of the populations across the landscape, and connectivity among populations. In order to achieve redundancy, the distribution of picture-wing fly populations across their geographic range should exceed the area of impact of a catastrophic event that would otherwise overwhelm the resilience of the populations. Essentially, the more populations of *D. neoclavisetae* and the broader the distribution of those populations, the more redundancy the species will exhibit, thereby increasing its ability to survive a catastrophic event. Captive populations of a species would provide a source of individuals that could supplement this redundancy. For *D. neoclavisetae*, redundancy requires the presence of multiple, stable to increasing populations distributed across its wet montane forest range between the elevations of 3,400 and 4,600 ft (1,040 and 1,400 m) where there is abundant host substrate.

Representation is the ability of *Drosophila neoclavisetae* to adapt to changing environmental conditions over time and can be measured by having one or more populations of a species occupying the full range of suitable habitat used by the species. Alternatively, representation can be viewed as maintaining the breadth of genetic diversity, within and among, populations. This allows the species to adapt to changing environmental conditions over time. The mobility of *D. neoclavisetae* provides a means of connecting nearby populations to support some genetic exchange and representation. However, connectivity is determined by the distance that can reasonably be achieved by the picture-wing fly, which is estimated at 100 meters (Science Panel 2005, p. 5) and is severely hampered by fragmentation of suitable habitats with host substrate and limited occurrence of *D. neoclavisetae*.

Mate preference studies suggest sexual selection plays a critical role in genetic differentiation during founder events. According to Kaneshiro (2004, entire; Kaneshiro 2020, pers. comm., entire) females that are highly discriminating in mate selection, under conditions of small population size, may not encounter males that are able to satisfy their courtship requirements. Small population size can result in strong selection for less discriminating females resulting in genetic recombination and a shift of gene frequencies toward the genotypes of less discriminating females within a few generations (Kaneshiro 2004, p. 45). Kaneshiro (2004, p. 43) states:

“Under these circumstances, the population is now provided with a genetic milieu where previously co-adapted gene complexes become available for recombination. Novel recombinants may be generated which provide the genetic variability required for the founder population to adapt to the new habitat or environment. Thus, the dynamics of sexual selection can be a synergist for species formation.”

With such a small gene pool and the intense inbreeding, genetic drift occurs resulting in changes within the group. Males born into this group may not have the right courtship maneuvers to satisfy the females. The population may die off from a lack of mating. But at this point, in the early stages of the founder event, the non-discriminating females may support population growth because they mate with the males despite their “poor” courtship performance. Essentially, the non-discriminating females may save the day (Kaneshiro 2004, pp. 43-45)

There is much evidence that genetic variability may be maintained even when populations are subjected to drastic reduction in size (Kaneshiro 1988, entire, Kaneshiro 2004, entire; Kaneshiro 2006a, entire). Based on Hawaiian *Drosophilidae* studies, in most cases where populations have been subjected to reduction in size due to environmental stress, the species still has the genetic capacity to generate novel genetic recombinants via differential sexual selection (Kaneshiro (2004, p. 49). Sexual selection concepts would also apply to captive breeding programs, which could help maintain genetic variability. Thus, the demographics of small populations and the genetic consequences of reduced population size as well as the dynamics of the sexual selection system are extremely important aspects of the evolutionary process and genetic recombination events.

These traits would potentially lead to and sustain a new population, in the presence of suitable host substrate and the absence of threats. The ability of a population to expand within an occupied site is constrained by the size of the area, availability of suitable oviposition and larval host material, and condition (e.g. humidity) of the habitat that surrounds the occupied site. However, the ability of *Drosophila neoclavisetae* to locate distant resources and colonize new areas is impeded by the following: 1) the rarity of the picture-wing fly populations 2) uneven distribution or isolation of suitable host substrate either temporally or spatially, 3) the presence of threats to the extant populations of the picture-wing fly and its host, and 4) the lack of connectivity or amount of distance between suitable host plants and picture-wing fly populations.

FACTORS INFLUENCING VIABILITY

Threats

Habitat Loss and Degradation

The primary factors that pose serious and ongoing threats include: (1) low number of populations and individuals based on lack of presence during surveys of 95 percent of the habitat of *Drosophila neoclavisetae* from the 1980s to 2005; (2) predation by nonnative wasps and ants; (3) competition for breeding resources; (4) parasitization from nonnative wasps; (5) habitat and plant host degradation and destruction by nonnative ungulates, rats, and nonnative plants; (6) drought; (7) increased risk of fire; (8) inadequate regulatory mechanisms to address nonnative species; (9) catastrophic natural disasters; (10) potential climate change, and the interaction of these threats.

Ungulates

Drosophila neoclavisetae is likely dependent on decaying bark or stems of *Cyanea* spp. for oviposition and larval development (Kaneshiro and Kaneshiro 1995, p. 20; Montgomery 1975, p.83; Science Panel 2005, p. 26; Magancca 2008, entire). These plant species are short-lived perennial shrubs and are highly susceptible to damage from nonnative ungulates, such as feral pigs (*Sus scrofa*), that occupy the wet montane habitat (Foote and Carson 1995, pp. 369–371; Kaneshiro and Kaneshiro 1995, entire; Science Panel 2005, p. 26; Magnacca et al. 2008, p. 32; Magnacca 2012a in litt., entire; Science Panel 2005, pp. 1–23). Pig rooting and herbivory can cause lack of regeneration of picture-wing fly host plants due to destruction of seedlings and consumption of fruit and seeds (Magnacca et al. 2008, p. 32). As a result, the host plants decrease throughout their ranges in areas that are not fenced and ungulate-free. Browsing by ungulates also alters the essential microclimate in picture-wing fly habitat by opening the understory. This can lead to increased desiccation of soil and host plants and disruption of the host plants' life cycle and decay processes. This subsequently disrupts the picture-wing fly's life cycle, particularly oviposition and the availability of substrate for the larval development (Magnacca et al. 2008, pp. 1, 32). Foote and Carson (1995), found that pig exclosures on Hawai'i supported significantly higher relative frequencies of picture-wing flies and native host plants compared to other native and nonnative *Drosophila* species (7 percent of all observations occurred outside of the exclosure and 18 percent of all observations occurred inside the exclosure).

Invasive Nonnative Plants

In addition to direct damage to the picture wing fly hosts, ungulates disperse nonnative seeds and create open, disturbed areas conducive to weedy plant invasion and establishment of nonnative plants. Nonnative plants adversely affect microhabitat by modifying the availability of light, shifting soil-water regimes, changing nutrient cycling processes, altering fire characteristics of native plant habitat, outcompeting natives, and inhibiting the growth of native plant species (Vitousek 1987, p. 224). This results in the conversion of a community dominated by native vegetation to one dominated by nonnative vegetation not supportive of *Drosophila neoclavisetae* survival (Cuddihy and Stone 1990, p. 74; Vitousek 1992, pp. 33–35).

The picture-wing fly's likely host plants (*Cyanea* spp.) are highly susceptible to competition from nonnative plants. The most significant nonnative plants in the picture-wing flies habitat appear to be *Psidium cattleianum* (strawberry guava), *Clidemia hirta* (Koster's curse), and *Tibouchina herbacea* (glory bush) (Bartlett, 2006, in litt., entire; Foote and Carson 1995, pp. 370–37; Starr et al. 2003, p. 3; Science Panel 2005, entire). This conversion has negative impacts on the host plants that the picture-wing fly depends for essential life-history needs and creates opportunities for subsequent establishment of nonnative vertebrates and invertebrates.

Though less impact has occurred in the wet montane habitats of *Drosophila neoclavisetae*, Maui has experienced prolonged periods of abnormally dry to extreme drought conditions for the past 10 to 25 years (NIDIS 2020). This has resulted in overall habitat degradation and appears to alter decay processes of the picture-wing fly host plants. Drought also alters the entire plant community on which the fly depends. 'Ōhi'a is an important tree in the wet montane habitat of *D. neoclavisetae* and is susceptible to prolonged drought (Magnacca 2012a in litt., entire).

Drosophila neoclavisetae prefers the dense shade in the fern understory, which is negatively impacted by the lack of dense and continuous overstory.

Fire

Though wet forest habitat of *Drosophila neoclavisetae* are generally at less risk of fire than mesic habitats, fire does pose a threat to the picture-wing fly because individuals or their habitat range are located in or near areas at risk for fire due to severe drought or the presence of highly flammable nonnative grasses and shrubs. Human alteration of landscapes and the introduction of nonnative plants, especially grasses, has led to greater frequency, intensity, and duration of fires (Brown and Smith 2000, p. 172). Grass-fueled fire often kills most native trees and shrubs (D'Antonio and Vitousek 1992, p. 70, 73-74). Extreme drought conditions are also contributing to the number and intensity of the wildfires on Maui which can penetrate into the wet forest (USFWS 2013a, p. 64663-64664). The ongoing drought in the adjacent mesic forests results in an increasing accumulation of dead trees, which increases the fuel load and threat of wildfires in the area. The extraordinary amount of dead wood accumulation in this habitat means any fire that occurs there likely would be extremely damaging.

Predation, Competition, and Disease

The western yellow-jacket wasp (*Vespula pensylvanica*) is an aggressive, generalist predator that threatens *Drosophila neoclavisetae* (Gambino et al. 1987, p. 170). This nonnative social wasp species was first reported from O'ahu in the 1930s. An aggressive race became established in 1977 (Gambino et al. 1987, p. 170). This species is now particularly abundant between 1,969 and 5,000 ft (600 and 1,524 m) in elevation throughout the state (Gambino et al. 1990, pp. 1,088-1,095; Foote and Carson 1995, p. 371). It is widespread in the wet montane habitat of Maui from which *D. neoclavisetae* is known. In temperate climates, the yellow-jacket wasp has an annual life cycle; in Hawai'i, colonies often persist through a second year. This allows them to have larger numbers of individuals and thus, a greater impact on prey populations (Gambino et al. 1987, pp. 169-170). The wasps have been observed carrying and feeding upon recently captured adult Hawaiian *Drosophila* (Kaneshiro and Kaneshiro 1995, pp. 40-45). *D. neoclavisetae* may be particularly vulnerable to predation by wasps due to their conspicuous lekking behavior (male territorial defensive displays with other males) and courtship displays that can last for several minutes (Kaneshiro 2006a p. 4-5; Kaneshiro 2006b in litt., entire). The concurrent arrival of the western yellow-jacket wasp and decline of picture-wing fly observations in some areas suggest that the wasp may have played a significant role in the decline of some populations (Carson 1986, pp. 3-9; Foote and Carson 1995, p. 371; Kaneshiro and Kaneshiro 1995, pp. 40-45; Science Panel 2005, pp. 1-23).

The number of native parasitic Hymenoptera (parasitic wasps) in Hawai'i is limited, and only species in the family Eucilidae are known to use Hawaiian picture-wing flies as hosts (Montgomery 1975, pp. 74-75; Kaneshiro and Kaneshiro 1995, pp. 44-45). However, several species of small parasitic wasps (Family Braconidae), including *Diachasmimorpha tryoni*, *Diachasmimorpha longicaudata*, *Opius vandenboschi*, and *Biosteres arisanus*, were purposefully introduced into Hawai'i to control nonnative pest tephritid fruit flies (Funasaki et al. 1988, pp. 105-160). These parasitic wasps are also known to attack other species of flies, including native flies in the family Tephritidae. While these parasitic wasps have not been recorded parasitizing Hawaiian picture-wing flies and, in fact, may not successfully develop in Drosophilidae, females

will indiscriminately sting any fly larvae in their attempts to oviposit, resulting in mortality of the larvae (USFWS 2013a, p. 64676). Because of this indiscriminate predatory behavior, nonnative parasitoid wasps represent a threat to *Drosophila neoclavisetae*.

In 2014, an ichneumonid wasp, *Gelis tellenae*, emerged from what was believed to be a healthy, intact *Drosophila mulli* pupa (Magnacca 2014 in litt., entire). The parasitized pupa was collected from the Upper Waiākea Forest Reserve on the island of Hawai‘i. It is not known at this time how widespread the parasitoid wasp is or the extent of parasitization of other picture-wing fly species, such as *Drosophila neoclavisetae* on Maui.

Picture-wing flies evolved in the absence of predation pressure from ants. Ants can be particularly destructive predators because of their high densities, recruitment behavior, aggressiveness, and broad range of diet (Reimer 1993, pp. 13-17). They can prey directly upon the eggs and larvae of the picture-wing flies or exclude the flies through interference or competition for breeding resources or shelter sites (Krushelnysky et al. 2005, p. 6). The threat of ant predation on *Drosophila neoclavisetae* is amplified by the fact that most ant species have winged reproductive adults (Borror et al. 1989, p. 738) and can quickly establish new colonies in additional locations (Staples and Cowie 2001, p. 55). These attributes allow some ants to destroy otherwise geographically isolated populations of native arthropods (Nafus 1993, pp. 19, 22-23). All ant species occurring in Hawai‘i are nonnative (Perkins 1913, p. xxxix) and at least four particularly aggressive species threaten *D. neoclavisetae*. Big-headed ants (*Pheidole megacephala*), long-legged or yellow crazy ants (*Anoplolepis gracilipes*), Papuan thief ants (*Solenopsis papuana*), and Argentine ants (*Linepithema humile*), are aggressive, generalist predators (preying on a variety of species) that have severely impacted the native insect fauna (Krushelnysky and Gillespie 2010, pp. 643-655; Reimer 1993, pp. 13-17). As a group, ants occupy most of Hawaii’s habitat types, from coastal to subalpine ecosystems; however, many species are still invading mid-elevation montane mesic forests. Few species have been able to colonize undisturbed montane wet ecosystems (Reimer 1993, pp. 13-17). Ants are more likely to occur in high densities in the mesic habitats than the wet montane habitat occupied by *D. neoclavisetae* (Reimer 1993, pp. 13-17). The big-headed ant is considered one of the most invasive and widely distributed ants in the world (Holway et al. 2002, pp. 181-233; Krushelnysky et al. 2005, p. 5). In Hawai‘i, big-headed ants are the most common ants found from dry coastal to lowland mesic habitat, though it has been found in some dry and mesic habitats up to 4,000 ft (1,219 m) in elevation (Reimer 1993 p. 14). Although *D. neoclavisetae* is known from wet montane habitats, populations that occur near the edge or in the transition zone from mesic to wet montane could be at risk. With few exceptions, native insects have been eliminated in habitats where big-headed ants are present (Perkins 1913, p. xxxix; Gagne 1979, pp. 80-81; Gillespie and Reimer 1993, p. 22). Consequently, big-headed ants represent a threat to the picture-wing fly, in their habitats (Reimer 1993, pp. 14, 17; Holway et al. 2002, pp. 181-233; Krushelnysky et al. 2005, p. 5). Yellow crazy ants appeared in Hawai‘i in 1952, and now occur on Hawai‘i, Kaua‘i, Maui, and O‘ahu (Reimer et al. 1990, p. 42; AntWeb. Version 8.33, 2020). In general, the species inhabits low-to-mid-elevations (less than 2,625 ft (800 m)), but the ants have been collected as high as 3,938 ft (1,200 m) at Haleakala National Park (Medeiros et al. 1986, entire; Reimer et al. 1990, p. 42). Although surveys have not been conducted to ascertain this species’ presence in areas last known to be occupied by *D. neoclavisetae*, yellow crazy ants penetrate into the wet rain forests in the lowlands (Reimer 1993, p. 15). Due to the ant’s

aggressive nature and ability to spread and colonize new locations, the ant poses a risk to the picture-wing fly, should the ant colonize the habitats occupied by the picture-wing fly. Yellow crazy ants can form high densities, or super colonies in a relatively short period of time with serious consequences for picture-wing flies (Gillespie and Reimer 1993, p. 21; Lester and Tavite 2004, p. 391).

The Papuan thief ant is the only abundant, aggressive ant that has invaded intact mesic to wet forest, as well as coastal and lowland dry habitats. This species occurs on the main Hawaiian Islands, and is still expanding its range (Reimer 1993, p. 14). Because of the ant's expanding range and its widespread occurrence that overlaps with the habitat of *Drosophila neoclavisetae*, the ant species is a threat to the picture-wing fly throughout its range. The Argentine ant was first noted on the island of O'ahu in 1940; now the ants are established on all the main Hawaiian Islands (Reimer et al. 1990, p. 42). Argentine ants do not disperse by flight, instead colonies are moved about with soil and construction material. The Argentine ant is found from coastal to subalpine ecosystems on the island of Maui, and on the slopes of Mauna Loa in the mesic ecosystems on Hawai'i (Krushelnycky and Gillespie 2010, pp. 643-655). The Argentine ant has been documented to reduce or eliminate populations of native arthropods in Haleakalā National Park on Maui (Cole et al. 1992, pp. 1313-1322). Argentine ants severely impact larval stages of many flies; they are significant predators on pest fruit flies and are associated with the decline of an endemic phorid fly (*Megaselia* sp.) (Krushelnycky and Gillespie 2010, pp. 643-655). Though there is no documented occurrences of predation by Argentine ants on picture-wing flies, these ants are considered to be a threat to native arthropods located at higher elevations (Cole et al. 1992, pp. 1313-1322; Science Panel 2005, pp. 1-23; USFWS 2013a, pp. 64677-64678).

Coqui frogs, *Eleutherodactylus coqui*, were introduced to the State of Hawai'i in the late 1980s (Woolbright et al 2006) and are present on Maui. On Maui, populations of frogs are known in and around nurseries and hotels, residential areas and several large natural area populations (Maui Invasive Species Committee 2020, entire). They have limited predators (mongoose, rats, and feral cats) enabling these frogs to become successful invaders across wet forest habitats and allowing their populations to grow extraordinarily dense compared to in their native habitat of Puerto Rico (Woolbright et al. 2006, entire). The spread to higher elevations poses a threat to *Drosophila neoclavisetae*. An analysis of coqui frog diets at lowland sites on the islands of Hawai'i and Maui found many invertebrates consumed by the frogs were leaf litter insects, however, a large number of flying insects were also present, indicating that these frogs are actively foraging while climbing trees (Beard 2007, p. 289). Most individual frogs are found within 6 to 10 ft (2.0 to 3.0 m) from the forest floor, with few found higher in the canopy (Beard et al. 2003, entire). If conditions are wet, all size classes of the frog forage higher off the ground, therefore allowing for aerial insect consumption to be more likely. Dietary analysis of the coqui frog on the island of Hawai'i showed that aerial insects make up 33.8 percent of the diet (Bernard & Mautz 2016, pp. 3413-3416). The frogs have the ability to consume 4500-56,000 prey/hectare/night, with 1,500-19,000 of these being aerial insects (Bernard & Mautz 2016, pp. 3413-3414). Dipterans, the soft bodied insect order that includes the picture-wing flies, represented 1.21 percent of the frog stomach content at lower elevations. Coqui frog is not known to be established in the Pu'u Kukui area at this time.

Picture-wing flies are adversely impacted by competition with several species of nonnative tipulid flies (large crane flies) in the family Tipulidae, for larval host substrates. The larvae of nonnative tipulid flies feed within the decomposing bark of the host plants likely utilized by *Drosophila neoclavisetae* (Science Panel 2005, pp. 1–23; Magnacca 2005 in litt., entire). The effect of tipulid competition is a reduction of available host plant material for the larvae of the picture-wing fly. In laboratory studies, Grimaldi and Jaenike (1984, pp. 1113–1120) demonstrated that competition between *Drosophila* spp. larvae and other fly larvae can exhaust food resources, which affects both the probability of larval survival and the body size of adults, resulting in reduced adult fitness, fecundity, and lifespan. It has been suggested that several species of soldier flies and neriid flies also impose a similar threat to Hawaiian picture-wing flies (Science Panel 2005, pp. 1–23).

The plant disease, rapid ‘ōhi‘a death (ROD) is an ongoing threat to ‘ōhi‘a, an important canopy tree in the wet montane habitats. This lethal disease of ‘ōhi‘a is caused by two fungal pathogens, *Ceratocystis lukuohia* and *C. huliohia* (Barnes et al., 2018, entire). *C. huliohia* is less aggressive and has been found on the islands of Kaua‘i, O‘ahu, Maui, and Hawai‘i; *C. lukuohia* is highly aggressive and has been identified on the islands of Hawai‘i and Kaua‘i (Friday et al. 2020, entire; Heller et al. 2019, entire). In July 2019, a single ‘ōhi‘a tree was confirmed to be infected with *C. huliohia* on Maui and that tree was destroyed. Hundreds of thousands of ‘ōhi‘a have died from this fungus infection on the island of Hawai‘i, but the severe pathogen has not yet established on Maui (Friday et al. 2020, entire). Should *C. lukuohia* become established in the wet habitat of *Drosophila neoclavisetae* it would have similar impacts as drought. Like drought, the loss of canopy allows more sunlight to reach the forest floor increasing the temperature and lowering the humidity, and subsequently, adversely affecting the picture-wing fly and its habitat.

Several species of nonnative rats, including the Polynesian rat (*Rattus exulans*), the roof rat (*Rattus rattus*), and the Norway rat (*Rattus norvegicus*) are present on the Hawaiian Islands and cause considerable environmental degradation (Staples and Cowie 2001). The seeds, bark, and flowers of the picture-wing flies’ host plants, *Cyanea* spp., are susceptible to grazing by all the rat species (Science Panel 2005; USFWS 2006a, p. 26843) The grazing by rats causes host plant mortality, diminished vigor, and seed predation, resulting in reduced host plant fecundity and viability (Science Panel 2005; USFWS 2006a, p. 26843).

Insufficient Regulatory Mechanisms

Existing State and Federal regulatory mechanisms are not effectively preventing the introduction and spread of nonnative species from outside the State of Hawai‘i or within the State between islands and watersheds. Predation by nonnative invertebrate species such as introduced wasps and ants, parasitization by nonnative introduced wasps, and habitat-altering, nonnative plant species and ungulates pose major ongoing threats to the picture-wing fly. The State’s current management of nonnative game mammals is inadequate to prevent the degradation and destruction of habitat of the picture-wing fly.

Nonnative feral ungulates pose a threat to *Drosophila neoclavisetae* through destruction and degradation of the species’ habitat and herbivory of its host plants. Regulatory mechanisms are inadequate to address this threat (USFWS 2006a, p. 26842–26844). The State of Hawai‘i provides game mammal (feral pigs and goats, and axis deer) hunting opportunities on State-

designated public hunting areas on the island of Maui (HDLNR 2015, pp. 19–21 and 66–77). The State’s management objectives for game animals range from maximizing public hunting opportunities to support sustained yield in some areas to completely removing game animals by State staff, or their designees, in other areas (HDLNR 2015, entire).

Currently, four agencies are responsible for inspection of goods arriving in Hawai‘i (USFWS 2006a, pp. 26846–26848; USFWS 2013a, p. 64679). The Hawai‘i Department of Agriculture inspects domestic cargo and vessels and focuses on pests of concern to Hawai‘i, especially insects or plant diseases not yet known to be present in the State. The U.S. Department of Homeland Security’s Customs and Border Protection is responsible for inspecting commercial, private, and military vessels and aircraft and related cargo and passengers arriving from foreign locations (USFWS 2006a, pp. 26846–26848; USFWS 2013a, p. 64679). The U.S. Department of Agriculture-Animal and Plant Health Inspection Service-Plant Protection and Quarantine inspects propagative plant material, provides identification services for arriving plants and pests, and conducts pest risk assessments among other activities (USFWS 2006a, pp. 26846–26848; USFWS 2013a, p. 64679–64680). The Service inspects arriving wildlife products, enforces the injurious wildlife provisions of the Lacey Act (18 U.S.C. 42; 16 U.S.C. 3371 et seq.), and prosecutes CITES (Convention on International Trade in Wild Fauna and Flora) violations (USFWS 2006a, pp. 26846–26848; USFWS 2013a, p. 64680). The State of Hawai‘i allows the importation of most plant taxa, with limited exceptions (USFWS 2006a, pp. 26846–26848; USFWS 2013a, p. 64680). It is likely that the introduction of most nonnative invertebrate pests to the State has been and continues to be accidental and incidental to other intentional and permitted activities. Many invasive weeds established on Maui have currently expanding ranges. Resources available to reduce the spread of these species and counter their negative ecological effects are limited. Control of established pests is largely focused on a few invasive species that cause significant economic or environmental damage to public and private lands, and comprehensive control of an array of invasive pests remains limited in scope (USFWS 2006a, pp. 26846–26848; USFWS 2013a, p. 64680–64681).

Biological Limitations

Like most native island biota, the Hawaiian picture-wing fly is particularly sensitive to disturbances due to low number of individuals. The species has only been observed two times, 1969 and 1975 in upper West Maui despite surveys of 95 percent of its habitat range (Service Panel 2005, entire; Magnacca 2019 in litt., entire). Based on the rarity of observing the species, it is inferred that the species likely has very low numbers and one or two extremely small populations. Given the species’ relatively small geographic range that contains suitable hosts, *Drosophila neoclavisetae* is extremely vulnerable to extinction due to threats associated with low number of individuals

Because of limited numbers of individuals and only one population observed in the last 50 years (1975), a single catastrophic event (e.g., hurricane, drought, fire) may result in extirpation of the extant population and extinction of this species. Species with few known locations, such as *Drosophila neoclavisetae*, are less resilient to threats that might otherwise have a relatively minor impact on widely distributed species. For example, the reduced availability of host plant substrate or an increase in predation of the picture-wing fly adults that might be absorbed in a widely distributed species could result in a significant decrease in survivorship or reproduction

of a species with limited distribution. The limited numbers and only one population observed in the last half century magnifies the severity of the impact of the other threats.

Environmental Factors

Stochastic events such as hurricanes and high winds from severe storms and flooding-associated landslides can result in the direct loss of *Drosophila neoclavisetae* individuals and decimate an entire population (Carson 1986, p. 7; Foote and Carson 1995, pp. 369–370). High winds can also dislodge fly larvae from their host plants, destroy host plants, and expose the fly larvae to predation by nonnative yellow-jacket wasps (Carson 1986, p. 7; Foote and Carson 1995, p. 371).

Changes in environmental conditions that may result from global climate change include increasing temperatures, decreasing precipitation, and increasing storm intensities. The habitat of *Drosophila neoclavisetae* are likely to be affected by changes in temperature, humidity, precipitation and the frequency and severity of storms (Clark et al. 2020, entire). These stressors may change the habitats on Hawai‘i and exacerbate other threats making the habitats unsuitable for the *D. neoclavisetae*, its host plants, or both. Climate change vulnerability is defined as the relative inability of a species to display the possible responses necessary for persistence under climate change (changes in rainfall and temperatures). Based on Fortini et al. (2013, p. 70) an assessment of the likely host plants concluded that the *D. neoclavisetae* larval host species vary in vulnerability to the impacts of climate change. On a scale of 0 being not vulnerable to 1 being extremely vulnerable to climate change, *Cyanea kunthiana* and *C. macrostegia* had vulnerability scores of 0.594 and 0.419, respectively. Additionally, changes in temperature and humidity may alter the decay cycle of the host plant substrates the picture-wing flies require in order to breed.

Conservation Actions

Endangered Species Act

In 2006, the Service determined endangered status under the Endangered Species Act of 1973 (Act), as amended, for 12 species of picture-wing flies from the Hawaiian Islands including *Drosophila neoclavisetae* (USFWS 2006a, entire). The primary purpose of the Act is the conservation of endangered and threatened species and the ecosystems upon which they depend. The goal of such conservation efforts is the recovery of these listed species, so that they no longer need the protective measures of the Act. Conservation measures provided to species listed as endangered or threatened under the Act include recognition of threatened or endangered status, recovery planning, requirements for Federal protection, and prohibitions against certain activities. The Act encourages cooperation with the States and requires that recovery actions be carried out for all listed species. The Act and its implementing regulations in addition set forth a series of general prohibitions and exceptions that apply to all endangered wildlife and plants. For plants listed as endangered, the Act prohibits the malicious damage or destruction on areas under Federal jurisdiction and the removal, cutting, digging up, or damaging or destroying of such plants in knowing violation of any State law or regulation, including State criminal trespass law. Certain exceptions to the prohibitions apply to agents of the Service and State conservation agencies. The Service may issue permits to carry out otherwise prohibited activities involving endangered or threatened wildlife and plant species under certain circumstances. For endangered plants, a permit must be issued for scientific purposes or for the enhancement of propagation or survival. For federally listed species unauthorized collecting, handling, possessing, selling, delivering, carrying, or transporting, including import or export across State lines and

international boundaries, except for properly documented antique specimens of these taxa at least 100 years old, as defined by section 10(h)(1) of the Act, is prohibited. In addition, damaging or destroying any of the listed species is violation of the Hawai'i State law prohibiting the take of listed species. The State of Hawai'i's endangered species law (HRS, Section 195-D) is automatically invoked when a species is Federally listed, and provides supplemental protection, including prohibiting take of listed species and encouraging conservation by State government agencies. *D. neoclavisetae* is known from State lands and private lands (USFWS 2008, entire).

Critical Habitat

In 2008, critical habitat totaling 134 ac (54 ha) were designated for *Drosophila neoclavisetae* on West Maui and went into effect January 9, 2009 (USFWS 2008, entire). Ranging in elevation between 3,405 to 4,590 ft (1,040 to 1,400 m), this critical habitat unit occurs within the boundary of the Pu'u Kukui Watershed Preserve and is State-owned. The area has populations of *Cyanea kunthiana* and *C. macrostegia*.

Fencing and Host Plants

Fencing, ongoing pig control and invasive plant management in the Pu'u Kukui Watershed lands has decreased the negative impacts to *Cyanea* spp. and other native fauna (Science Panel 2005 pp. 19–20; Bartlett 2006 in litt., entire).

CURRENT CONDITION

Historic Condition

Pre-Human Habitat and Species Distribution

Geologic-informed modeling was used to evaluate the relationship between changing island area and Hawaiian *Drosophila* species richness for the Hawaiian archipelago during island formation and evolutionary radiation (forming of new species) (Lim and Marshall 2017, p. 3). Results infer that the formation of new picture-wing fly species, in general, has slowed on the island of Hawai'i, but has not yet become negative where no speciation occurs due to species saturation of ecological niches. This contrasts with speciation in Maui Nui (the islands of Moloka'i, Maui, Lāna'i, and Kaho'olawe) and O'ahu where model results infer that the formation of species has ceased or entered a negative value (Lim and Marshall 2017, p. 2).

In general, picture-wing fly adaptive radiations on older islands (northwestern islands) are characterized by initially increasing rates of species accumulations, followed by a slowdown in the rate of speciation, and eventual decline of the number of species (Lim and Marshall, p. 3). Based on evolutionary modeling, on Maui Nui and O'ahu, the time of evolutionary expansion is long past, and they are now undergoing long-term evolutionary decline in speciation rate. On Kaua'i, the oldest island, modeling of picture-wing fly suggests species formation has slowed down and may be entering evolutionary decline in the number of species (Lim and Marshall 2017, p. 3).

Magnacca et al. (2015, entire) estimated that O'ahu was available for colonization around 3.5 million years ago, Maui Nui was available for colonization around 2.0 million years ago and the island of Hawai'i was available around 0.8 million years ago. The ancestor of *Drosophila neoclavisetae* and the closely related *D. clavisetae* from East Maui was most likely from an older

island (Magnacca et al 2015, p. 234). Immediately prior to human arrival to Maui, speciation had likely slowed or stopped (Lim and Marshall 2017, entire).

The adaptive radiation of the *Drosophila* species likely followed the availability of ecological niche development. A continuous availability of suitable plant host substrate would need to be present for oviposition and larval development for continued generational and population success. *Drosophila neoclavisetae* larvae are most likely dependent on a limited number of plant species—and perhaps other closely related species in existence at the time—thus, the host plant would have to be well established and present in all ages and stages. Adults are generalist microbivores, but the persistence of the picture-wing fly species is closely linked to availability of their breeding substrate. The species may have occurred wherever the host plants and necessary humidity to support the fly occurred. It likely competed for or shared larval resources with other picture wing fly species, which use the same substrate and occupy the same habitat as *D. neoclavisetae* (Montgomery 1975, entire; Magnacca et al., 2008, p. 1). However, the picture wing fly species would not have been threatened by habitat loss from anthropomorphic activities, introduced predators, or competition from nonnative species.

Historic trends

Survey methods for *Drosophila* typically involve collecting larval host plant material and rearing the larvae to adulthood or using a bait to attract the picture-wing flies. Hawaiian *Drosophila* life cycles are influenced by rainfall patterns and other environmental variables, making survey results difficult to compare over time and across sites. Presence of even the most common species fluctuate widely, confounding negative survey records for a taxa (Magnacca 2012b in litt. 2012b). Historically, *Drosophila neoclavisetae* was rare, only observed once at two locations in relatively close proximity to one another in West Maui. The first collection of the species was by H.L. Carson in 1969 at Pu‘u Kukui near the 4,500 ft (1,372 m) elevation (Perreira and Kaneshiro 1990, p. 83). The females collected were used as the holotype for characterization of the species (Perreira and Kaneshiro 1990, p. 83). The species was not observed in extensive surveys conducted in 1971. In 1975, a male and female were collected near the Pu‘u Kukui trail around the 3,600 ft (1,097 m) elevation by K.Y. Kaneshiro (Figure 3) (Perreira and Kaneshiro 1990, p. 83; Kaneshiro 2005, entire). In 1997 and subsequent years, extensive surveys of likely *D. neoclavisetae* habitat were conducted, but the species was not located (Kaneshiro 2005, entire).

Current Condition

The species was last observed in 1975 in the wet montane ‘ōhi‘a forest of Pu‘u Kukui on West Maui (Perreira and Kaneshiro 1990, p. 83; Kaneshiro 2005, entire). Prior to that, the species was observed in 1969. Fewer than 10 individuals at the two locations have ever been documented (Science Panel 2005, p. 26; Kaneshiro 2005, entire) despite the presence of a stable population of *Cyanea* sp. that established in some areas after pig control and management began in the area in the early 2000s and later (Science Panel 2005, p. 20; Hansen 2006 in litt., entire). Because of the importance of *Drosophila neoclavisetae* within several evolution and genetics studies, concerted effort over a period of several years was made to locate the species (Kaneshiro 2006c in litt., p.2). Kaneshiro estimates 95 percent of the potential habitat of *D. neoclavisetae* had been surveyed during these efforts, but the species has not been observed (Kaneshiro 2006c in litt., p. 4).

SPECIES VIABILITY SUMMARY

Resiliency

Resiliency is the capacity of an individual or population to withstand stochastic disturbance events. We define resiliency for *Drosophila neoclavisetae* based on population size (abundance), population growth rate, and habitat quality. Rarely observed, *D. neoclavisetae* is only known from 2 general population areas on Maui. Fewer than 10 individuals have ever been observed; all occurring in 1969 and 1975, all in wet montane ‘ōhi‘a forest habitat at Pu‘u Kukui. For *D. neoclavisetae* to reproduce, a female needs to find a suitable male for mating. If a population is largely comprised of discriminating females and males that do not meet the discriminating females’ requirements for courtship and breeding, then breeding may bottleneck. The absence of observations over the last 50 years does not preclude the possibility that small, isolated populations are still extant where suitable habitat and the necessary host plant substrates are present, given the difficulty in assessing *Drosophila* spp. However, concerted survey efforts have focused on 95 percent of the possible habitat of *D. neoclavisetae* and the species has not been observed. Nothing is known about the demographics or rate of mating encounter of any extant individuals.

Habitat needs include the availability of suitable stages of the host plants, likely *Cyanea* spp. for oviposition and larval development. Breeding generally occurs year-round, but egg laying and larval development increase as the availability of decaying matter increases. For the picture-wing fly to breed year-round, it requires suitable decaying host substrate be continuously available. This requires a functioning decay cycle that is largely driven by the presence of high humidity, such as that which occurs during the rainy season. In addition to a constant supply of suitable larval substrate, the substrate needs to be available within a distance the picture-wing fly can navigate. One of the likely host plant species, *Cyanea kunthiana*, of this picture-wing fly, is itself endangered, and thus, rare. However, *C. macrostegia* is abundant and is considered stable in population number, though unevenly distributed across the habitat largely due to ongoing ungulate control in the habitat. Nonetheless, the susceptibility of these hosts to ungulate damage and decreasing habitat area that provides the humidity necessary to support the picture-wing fly, makes this picture-wing fly species vulnerable to extirpation.

Resiliency also depends on a suitable habitat free from threats. Pig control within the habitat of *Drosophila neoclavisetae* and its likely hosts, *Cyanea* spp. is ongoing, though ungulate management does not provide protection throughout the entire wet montane habitat of the picture wing fly and its hosts plants. *Cyanea* spp. are favorites of ungulates, especially pigs, and are therefore, very susceptible to grazing and consumption of the fruit and seeds. Regardless of fence status or ungulate control, the seeds, bark, and flowers of *Cyanea* spp. are susceptible to rat predation, reducing the abundance and natural recruitment of these plant species. Populations of the host plants and the picture-wing fly are vulnerable to fire and other stochastic or catastrophic events such as drought and hurricane. Extended drought is currently occurring on Maui in the areas from which *D. neoclavisetae* is historically known. The habitats also require active management to prevent degradation of the habitat by nonnative plants. The size of the picture-wing fly and the conspicuous lekking and courtship displays likely contribute to the picture-wing flies’ vulnerability to predation by western yellow-jacket wasps and other predatory wasps. Nonnative wasps may indiscriminately oviposit in *D. neoclavisetae* larva or pupa resulting in the

death of the picture-wing fly. The species is also vulnerable to nonnative ants that prey on eggs, larvae, and pupae. Because it is likely that *D. neoclavisetae* uses decaying bark as a substrate for its larvae, it is also vulnerable to competition from nonnative crane flies for suitable host plant substrate. A resilient population of *D. neoclavisetae* has abundant individuals in multiple, stable to increasing populations in the wild and high quality habitat. The extant population of this picture-wing fly, if it is not extirpated, has significant deficiencies in the basic characteristics of resiliency and the species is in extreme danger of extirpation.

Resiliency of *Drosophila neoclavisetae* is extremely low based on: 1) less than 10 individuals observed in 1969 and 1975; 2) vulnerability of the likely host plants to ungulates and rats; 3) presence of threats from nonnative species; 4) isolation of any extant populations from each other; and 5) imminent threat of extirpation and extinction by a stochastic event such as drought, fire, or hurricane.

Redundancy

Redundancy is the ability of *Drosophila neoclavisetae* to withstand catastrophic events and is measured by the presence of multiple, stable to increasing populations distributed across its historical wet montane range on Maui. The species has extremely limited spatial redundancy to nonexistent redundancy based on the most recent surveys in the late 1990s to the early 2000s. *D. neoclavisetae* appears to be limited to one general location on Pu'u Kukui. The chances of other populations occurring within suitable wet montane habitat on West Maui, is possible, but not likely, given that 95 percent of the habitat has been searched. If an extant undocumented population exists, it is likely to be functionally isolated if it does not have another population with which to breed. The historically known populations of the *D. neoclavisetae* make the species highly vulnerable to extinction due to a variety of natural processes such as drought or random catastrophes such as hurricanes (Lande 1988). As with the individual needs, persistence of populations and the species is closely linked to availability of their breeding hosts (Magnacca et al., 2008, p. 1). The picture-wing fly and its plant hosts are threatened by catastrophic changes to their habitat caused by drought, changes in humidity levels associated with changes in canopy cover from multiple causes, predation, parasitization, and competition for bark substrate. The species may have some temporal redundancy conveyed by its ability to breed year-round provided adequate humidity and host substrate is present, but limited numbers within an extant population likely negates that advantage. Based on these factors, *D. neoclavisetae* has extremely low to nonexistent redundancy.

Representation

Representation for *Drosophila neoclavisetae* to persist and adapt to changing environmental conditions over time would require having multiple stable to growing populations throughout West Maui in the species wet montane range. The species would need to possess the genetic diversity that comprises and defines the species. The existing biogeographical range of *D. neoclavisetae* delimits its genetic diversity. The amount of genetic diversity lost by the inferred severe reduction in abundance or extirpation at its historically documented locations is unknown. Distance between any extant isolated population likely impedes, if not completely precludes, genetic exchange among any existing individuals. The species typically travels only about 100 meters or so from its hosts. Portions of the suitable habitat is threatened by ungulates, rats, and stochastic events that affect West Maui. The complex breeding strategy of the Hawaiian picture-

wing fly species provides opportunities for selection and adaption to environmental stresses. It does not intrinsically provide for defense against predation or drought, other than what influences behavior. Despite the remarkable selection and breeding behaviors that support genetic recombination in founding events, the limit of the species to one population observed over 50 years ago and genetic isolation clearly make this species at risk of extirpation. With no representative breeding colonies in captivity and only one, highly at-risk population, the species has extremely low representation. Thus, the representation of *D. neoclavisetae* that is conferred by stable to increasing populations embodying the existing full genetic diversity being dispersed throughout its historical wet montane ‘ōhi‘a forest range on West Maui is extremely low.

Summary

The only populations of *Drosophila neoclavisetae* observed in the last 55 years (1969 and 1975) are from the Pu‘u Kukui area of West Maui (Kaneshiro 2005, entire; Kaneshiro 2006c in litt., entire). A population may still occur at Pu‘u Kukui, but the current status of that population is unknown. No observations of the species have been made at this location in 50 years, despite years of thorough surveys of 95% of the potential habitat for the species. If an extant population exists it is certainly isolated. If not already extirpated, the population on West Maui is at high risk of extirpation, which would lead to extinction of the species. Resiliency of the population is considered extremely low because of lack of abundance of individuals, lack of apparent growing populations, predation, parasitization, competition for larval substrate, and degradation of habitat quality from pigs, rats, and drought. The species has extremely low to nonexistent redundancy because the only two populations ever documented were last observed 50 to 55 years ago. Species representation is extremely low because the genetic diversity is seemingly limited to the species occurrence at Pu‘u Kukui or to an isolated, undocumented population. Distance between existing populations beyond 100 meters makes natural genetic exchange between any extant populations extremely unlikely. Therefore, the current viability of *D. neoclavisetae* is extremely low, if the species is extant (Table 1).

Table 1. Viability summary of *Drosophila neoclavisetae*, nalo mea hula, under current conditions.

Species	Resiliency	Redundancy	Representation	Viability
<i>Drosophila neoclavisetae</i>	Extremely low	Extremely low to nonexistent	Extremely low	Extremely low

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